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| <b>(51) International Patent Classification <sup>6</sup> :</b><br><b>A61K 31/05, 9/107, 47/18</b>  | <b>A1</b>   | <b>(11) International Publication Number:</b> <b>WO 96/29064</b><br><b>(43) International Publication Date:</b> 26 September 1996 (26.09.96) |
| <b>(21) International Application Number:</b> PCT/GB95/00579<br><b>(22) International Filing Date:</b> 17 March 1995 (17.03.95)<br><b>(71) Applicant:</b> ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).<br><b>(72) Inventors:</b> JONES, Christopher, Buchan; "Fernhurst", Yew Tree Close, Prestbury, Cheshire SK10 4HB (GB). PLATT, John, Henry; 9 Somerset Close, Congleton, Cheshire CW12 1SG (GB).<br><b>(74) Agent:</b> DENERLEY, Paul, Millington; Zeneca Pharmaceuticals, Intellectual Property Group, Intellectual Property Dept., Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). | <b>(81) Designated States:</b> AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TT, UA, UG, UZ, VN, ARIPO patent (KE, MW, SD, SZ, UG), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>With international search report.</i> |  |
| <b>(54) Title:</b> OIL IN WATER EMULSIONS CONTAINING PROPOFOL AND EDETATE<br><br><b>(57) Abstract</b><br><br>Pharmaceutical compositions containing 2,6-diisopropylphenol (propofol) are described for use as anaesthetics. A method for their preparation is described, as their use in producing anaesthesia including induction and maintenance of general anaesthesia and sedation.  |   |  |

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oil in water emulsions containing propofol and edetate

The present invention relates to 2,6-diisopropylphenol, known as propofol, and in particular to new pharmaceutical compositions containing propofol.

Propofol is an injectable anaesthetic which has hypnotic properties and can be used to induce and maintain general anaesthesia and for sedation for example in Intensive Care Units. Propofol is a highly successful anaesthetic and is marketed under the trademark 'Diprivan' for use in treating humans and under the trademark 'Rapinivet' for veterinary use.

Injectable anaesthetics, such as propofol, are administered directly into the blood stream. This gives rise to a rapid onset of anaesthesia influenced almost entirely by the rate at which the anaesthetic agent crosses the blood-brain barrier. It is therefore necessary for the anaesthetic agent to have sufficient lipid solubility to be able to cross this barrier and depress the relevant mechanisms of the brain. However highly lipid soluble molecules are generally poorly soluble in water and thus are difficult to formulate for intravenous injection. In some cases it may be possible to obtain a water soluble salt of the anaesthetic agent which releases a lipid soluble free base in vivo. This is not possible in many cases and, despite considerable research, it did not prove to be feasible with propofol. Thus it was necessary to conduct very substantial research and development into the formulation of propofol in order to obtain pharmaceutical compositions for administration to warm-blooded animals including humans.

The present applicants identified the anaesthetic properties of propofol and filed UK patent application no 13739/74 which was granted as United Kingdom Patent 1472793. Corresponding patents have been granted in the USA (USP 4056635, USP 4452817 and USP 4798846) and many other territories.

This patent claims inter alia a sterile pharmaceutical composition which comprises propofol in association with a sterile pharmaceutically-acceptable diluent or carrier the composition being suitable either directly or after dilution with a liquid diluent for

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parenteral administration to a warm-blooded animal.

In one aspect, UK 1472793 described the composition as preferably aqueous with propofol in sterile admixture with water and a surfactant or other solubilising agent. In another aspect the composition was described as aqueous with propofol in sterile admixture with water and an additional water-miscible, non-aqueous solvent. In a further aspect the composition was described as an oil-in-water emulsion in which propofol, either alone or dissolved in a water-immiscible solvent, is emulsified with water by means of a surfactant. In yet a further aspect the composition was described as a sterile solid or semi-solid mixture of propofol with a solid diluent, for example lactose, saccharin sodium or a cyclodextran which composition is suitable for dilution with a sterile aqueous diluent.

The patent describes many particular Examples of injectable compositions containing propofol including Examples with various surfactants, various solubilising agents, additional solvents, additional constituents (selected from stabilisers, preservatives and antioxidants), buffering agents and tonicity modifiers.

The present applicants conducted a wide range of studies to determine which type of formulation would be most appropriate for development to provide a formulation for marketing. After considerable effort a formulation of propofol and the surfactant Cremophor EL (Cremophor is a trade mark for a polyoxyethylene castor oil derivative) in water was selected. Cremophor EL was used as the carrier to solubilise the existing intravenous anaesthetic alphaxalone/alphadolone ('Althesin') and a modified form of Cremophor was used as the carrier to solubilise the intravenous anaesthetic propanidid ('Epontol').

The present applicants conducted a detailed series of studies in animals and ultimately administered the formulation to over 1000 humans. However, after about five or six years, anaphylactoid reactions were reported in a very small number of patients. Anaphylactoid reactions are allergic-type reactions. It was not clear that Cremophor EL had caused the anaphylactoid reactions in all instances but the present applicants concluded that an alternative formulation of propofol would have to be found and developed.

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A substantial amount of work on alternative formulations was performed and an oil-in-water emulsion was eventually selected for development. This was developed and in 1986 was launched in a number of markets under the trade mark 'Diprivan'. Since then this formulation has been launched in many markets throughout the world and propofol is highly successful being regarded by anaesthetists as a drug of great merit having unique qualities. In summary propofol is a short-acting anaesthetic, suitable for both induction and maintenance of general anaesthesia, for sedation to supplement regional analgesic techniques, for sedation of ventilated patients receiving intensive care and for conscious sedation for surgical and diagnostic procedures in Intensive Care Units. Propofol may be administered by single or repeated intravenous bolus injections or by continuous infusion. It is very rapidly removed from the blood stream and metabolised. Thus the depth of anaesthesia is easily controlled and patient recovery on discontinuing the drug is usually rapid and the patient is often significantly more clear headed as compared to after administration of other anaesthetics. Side-effects such as nausea and vomiting occur significantly less frequently following administration of propofol than following other general anaesthetic techniques such as with inhalational anaesthetics.

The present applicants have considered extending the range of propofol formulations in order to give the anaesthetist a wider armamentarium from which to select an appropriate drug. For example applicants have developed, as an alternative, an oil-in-water emulsion formulation of propofol wherein the concentration of propofol is twice that of the presently marketed drug.

In considering appropriate further formulations it is desirable to maintain the qualities that make 'Diprivan' of such merit, such as those aforementioned and provide a formulation with acceptable chemical and physical stability and which is readily manipulable by the anaesthetist or Intensive Care Unit (ICU) specialist.

An increasing proportion of the usage of 'Diprivan' is in the sedation of seriously ill patients particularly in Intensive Care Units and the like. In the sedation of such seriously ill patients

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administration of 'Diprivan' is typically by means of infusion. This requires the use of a 'giving set', which involves the linkage of a reservoir (typically a vial or syringe) of propofol, via appropriate tubing, to a luer connector and thence to a needle positioned in the patient's vein.

Microbial contamination of parenteral fluids used in 'giving sets' of this type has been recognised as one of many causes of nosocomial infection amongst ICU patients. Accordingly, for example in the USA, the general requirements of the Federal Food and Drug Administration (FDA) are that such 'giving sets' are changed frequently and in the case of 'Diprivan', it is required that the 'giving sets' are changed at least every 6 or 12 hours dependent on the presentation being used.

Intensive Care environments are busy and, as in other parts of the health services, there are pressures for cost-containment. The changing of 'giving sets' at least every 6 or 12 hours is relatively time-consuming for the highly skilled ICU nurse, Intensive Care Specialist or anaesthetist. This would particularly be the case when a number of seriously ill patients in an ICU are being infused at the same time.

Therefore, the applicants have sought to develop a new formulation of propofol which would enable 'giving sets' to be changed significantly less frequently (for example every 24 hours). This would be much more convenient for the nurse, Intensive Care Specialist or anaesthetist; would lower the pressure on staff, would result in fewer manipulations of 'giving sets' and may contribute to cost-saving in the ICU environment.

We have conducted substantial research and have found that the addition of small amounts of a selected agent to 'Diprivan' will enable the formulation to be administered in 'giving sets' that require changing significantly less frequently than is presently the case; in other words the time for administration and time between changes of the giving sets has been significantly improved. This increase in such times enables packs of increased size to be administered, increasing convenience for the users, decreasing wastage of 'Diprivan' and contributing to cost-containment.

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Furthermore, in the unlikely event of mishandling leading to accidental extrinsic contamination, the formulation will minimise the chance of microbial growth.

Our own UK Patent 1472793 discloses that formulations of propofol may optionally contain one or more additional constituents selected from stabilisers, preservatives and antioxidants, for example parabens derivatives, for example propyl p-hydroxybenzoate, butylated hydroxytoluene derivatives, ascorbic acid and sodium metabisulphite; metal ion sequestering agents, for example sodium edetate; and antifoaming agents, for example a silicone derivative, for example dimethicone or simethicone.

There is a difficulty in the addition of known preservatives to oil-in-water emulsions such as 'Diprivan'. As stated above, 'Diprivan' is an anaesthetic used for induction and maintenance of general anaesthesia and for sedation. The volumes administered can be considerable, particularly in the case of sedation. Accordingly, significant volumes of preservative may be administered to the patient receiving treatment. Thus very careful selection of additive must be made in order to satisfy drug Regulatory Authorities; particularly as the use of preservatives in single-dose, terminally sterilised, parenteral injectables is not suggested and/or is the subject of cautionary statements in various Guidelines, for example those of the US, UK and European Pharmacopeias.

Furthermore there is a particular problem in the inclusion of additives in an oil-in-water emulsion for parenteral administration. It is believed that for effectiveness, the antimicrobial properties of any preservative have to be exerted in the aqueous phase. Thus, a preservative with lipophilic properties incorporated at typical usage levels would not be effective as, although there would be some partitioning between the phases, there would be insufficient material in the aqueous phase. Increasing the overall quantity of such preservative would result in unacceptably high levels of preservative in the lipid layer leading to toxicity problems at least.

On the other hand, addition of a preservative with

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hydrophilic properties, eg an ionic material, also leads to problems. The addition of ionic material to an oil-in-water emulsion tends to destabilise the emulsion. With a higher ionic load (that is concentration of ionic material) the stabilising electrical charge (Zeta potential) on the oily droplets can change. Such electrical charge changes increase the probability of droplet collisions and increase the physical instability of the emulsion.

We studied the possibility of adding one of a number of antimicrobial agents to the oil-in-water emulsion. Such an agent would have to have no significant detrimental effect on the physical and chemical stability of the emulsion. Furthermore, such an agent would have to provide the antimicrobial activity being sought.

A number of potential agents were found to cause instability of the emulsion. Other potential agents failed to provide the level of antimicrobial activity being sought. In addition, we were seeking an agent that would provide these levels of activity at as low a concentration as possible in order to minimise the potential for physical instability and to minimise safety concerns.

After significant effort including consideration of the known preservatives phenylmercuric acetate, phenylmercuric nitrate, benzyl alcohol, chlorobutanol, chlorocresol and phenol and the study of the known preservatives sodium metabisulphite, sodium sulphite, sodium methyl hydroxybenzoate and sodium propyl hydroxybenzoate, we were unable to find a preservative that met our requirements. We then investigated the possible use of other agents which might have the action that we sought. We unexpectedly found that edetate, which is not regarded as a broad spectrum antimicrobial agent was the only agent that would meet our requirements. As referred to above, edetate as the sodium salt is mentioned in our UK Patent 1472793 as a possible metal ion sequestering agent. Sodium edetate is included in two of the many Cremophor-containing examples of that Patent.

Accordingly the present invention provides a sterile pharmaceutical composition for parenteral administration which comprises an oil-in-water emulsion in which propofol dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of



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edetate sufficient to prevent significant growth of microorganisms for at least 24 hours (in the event of adventitious, extrinsic contamination).

By an oil-in-water emulsion we mean a distinct two-phase system that is in equilibrium and in effect, as a whole, is kinetically stable and thermodynamically unstable. This is in complete contrast to a micellar formulation, for example with Cremophor EL, which is thermodynamically stable.

By the term "edetate" we mean ethylenediaminetetraacetic acid (EDTA) and derivatives thereof, for example the disodium derivative is known as disodium edetate. In general suitable edetates of this invention are those salts having lower affinity for EDTA than calcium. Particular derivatives of use in the present invention include trisodium edetate, tetrasodium edetate and disodium calcium edetate. The nature of the edetate is not critical, provided that it fulfils the function of preventing significant growth of microorganisms for at least 24 hours in the event of adventitious extrinsic contamination (e.g. preferably no more than 10-fold increase following a low level of extrinsic contamination, such as  $10 - 10^3$  colony forming units, at temperatures in the range of 20-25°C). As can be seen from the experimental section, sodium calcium edetate has some advantages over other additives but disodium edetate is exceptional. Accordingly, most preferably the edetate is disodium edetate.

Typically the edetate will be present in the compositions of the present invention in a molar concentration (with respect to the EDTA free acid) in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ . Preferably the edetate is present in the range  $3 \times 10^{-5}$  to  $7.5 \times 10^{-4}$ , for example in the range  $5 \times 10^{-5}$  to  $5 \times 10^{-4}$  and more preferably in the range  $1.5 \times 10^{-4}$  to  $3.0 \times 10^{-4}$ , most preferably about  $1.5 \times 10^{-4}$ .

A composition of the present invention typically comprises from 0.1 to 5%, by weight, of propofol. Preferably the composition comprises from 1 to 2% by weight of propofol and, in particular, about 1% or about 2%.

In another aspect of the invention propofol alone is emulsified with water by means of a surfactant. It is preferred that propofol is dissolved in a water-immiscible solvent prior to

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emulsification.

The water-immiscible solvent is suitably present in an amount that is up to 30% by weight of the composition, more suitably 5-25%, preferably 10-20% and in particular about 10%.

A wide range of water-immiscible solvents can be used in the compositions of the present invention. Typically the water-immiscible solvent is a vegetable oil, for example soy bean, safflower, cottonseed, corn, sunflower, arachis, castor or olive oil. Preferably the vegetable oil is soy bean oil. Alternatively, the water-immiscible solvent is an ester of a medium or long-chain fatty acid for example a mono-, di-, or triglyceride; or is a chemically modified or manufactured material such as ethyl oleate, isopropyl myristate, isopropyl palmitate, a glycerol ester or polyoxyl hydrogenated castor oil. In a further alternative the water-immiscible solvent may be a marine oil, for example cod liver or another fish-derived oil. Suitable solvents also include fractionated oils for example fractionated coconut oil or modified soy bean oil. Furthermore, the compositions of the present invention may comprise a mixture of two or more of the above water-immiscible solvents.

Propofol, either alone or dissolved in a water-immiscible solvent, is emulsified by means of a surfactant. Suitable surfactants include synthetic non-ionic surfactants, for example ethoxylated ethers and esters and polypropylene-polyethylene block co-polymers, and phosphatides for example naturally occurring phosphatides such as egg and soya phosphatides and modified or artificially manipulated phosphatides (for example prepared by physical fractionation and/or chromatography), or mixtures thereof. Preferred surfactants are egg and soya phosphatides.

The composition of the present invention is suitably formulated to be at physiologically neutral pH, typically in the range 6.0-8.5, if necessary by means of alkali such as sodium hydroxide.

The composition of the present invention may be made isotonic with blood by the incorporation of a suitable tonicity modifier for example glycerol.

The composition of the present inventions are typically sterile aqueous formulations and are prepared according to

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conventional manufacturing techniques using for example aseptic manufacture or terminal sterilisation by autoclaving.

The compositions of the present invention are useful as anaesthetics which includes sedation and induction and maintenance of general anaesthesia. Accordingly in another aspect the present invention provides a method of producing anaesthesia (including sedation and induction and maintenance of general anaesthesia) in a warm-blooded animal, including humans, which comprises administering parenterally a sterile aqueous pharmaceutical composition which comprises an oil-in-water emulsion in which propofol, either alone or in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant and which further comprises an effective amount of edetate.

Dosage levels of propofol for producing general anaesthesia, both induction (for example about 2.0-2.5 mg/kg for an adult) and maintenance (for example about 4-12 mg/kg/hr), and for producing a sedative effect (for example 0.3-4.5 mg/kg/hr), may be derived from the substantial literature on propofol. Furthermore the anaesthetist and/or physician would modify the dose to achieve the desired effect in any particular patient, in accordance with normal skill in the art.

The advantages referred to above for including edetate in propofol compositions apply also to intravenous fat emulsions which typically are administered, to patients in need thereof, over periods of a day or more. Intravenous fat emulsions (also known as parenteral nutrition emulsions) are administered, usually by infusion, to patients having requirements for additional calories and adequate nutrition, by oral or other means, is not desirable or is not possible. Intravenous fat emulsions typically maintain a positive nitrogen balance and provide an adequate source of energy (e.g. as fat), vitamins and trace elements. Such emulsions are used typically in intensive care environments but also in other hospital and domestic settings. Examples of such intravenous fat emulsions include Intralipid (marketed by Pharmacia), Lipofundin (Braun) and Travamulsion (Baxter). Intralipid, Lipofundin and Travamulsion are all trademarks.

Accordingly in another aspect, the present invention

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provides an intravenous fat emulsion which comprises an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours. In particular the present invention provides a sterile, aqueous composition for parenteral administration which comprises an oil-in-water emulsion in which a water-immiscible solvent is emulsified with water and stabilised by means of a surfactant and which further comprises an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours.

Furthermore, it has been proposed that various drugs may be administered in oil-in-water emulsions, for example see United States Patent 4168308. Accordingly in a further aspect, the present invention provides a sterile, aqueous composition for parenteral administration which comprises an oil-in-water emulsion containing a therapeutic or pharmaceutical agent, in which the agent, either alone or dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant and which further comprises an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours.

Suitable therapeutic or pharmaceutical agents are those capable of being administered parenterally in an oil-in-water emulsion. Typically such agents are lipophilic compounds and may for example be antifungal agents, anaesthetics, antibacterial agents, anti-cancer agents, anti-emetics, agents acting on the central nervous system such as diazepam, steroids, barbiturates and vitamin preparations. In particular the present invention relates to such oil-in-water emulsions which typically are administered, to patients in need thereof, over periods of a day or more.

Comments herein relating to typical and preferred propofol compositions of this invention and the preparation thereof apply mutatis mutandis to intravenous fat emulsions and to oil-in-water emulsions containing a therapeutic or pharmaceutical agent.

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**EXPERIMENTAL****Quantities:**

|                                 | <u>% (weight)</u> |
|---------------------------------|-------------------|
| propofol                        | 1.0               |
| soy bean oil                    | 10.0              |
| egg phosphatide                 | 1.2               |
| glycerol                        | 2.25              |
| disodium edetate dihydrate      | 0.0055            |
| (equivalent to disodium edetate | 0.005)            |
| sodium hydroxide                | q.s.              |
| Water for Injections            | to 100            |

**Preparation:**

All processing stages are carried out under Nitrogen and weights refer to weight in the final volume.

A sterile aqueous oil-in-water emulsion for parenteral administration is prepared as follows:

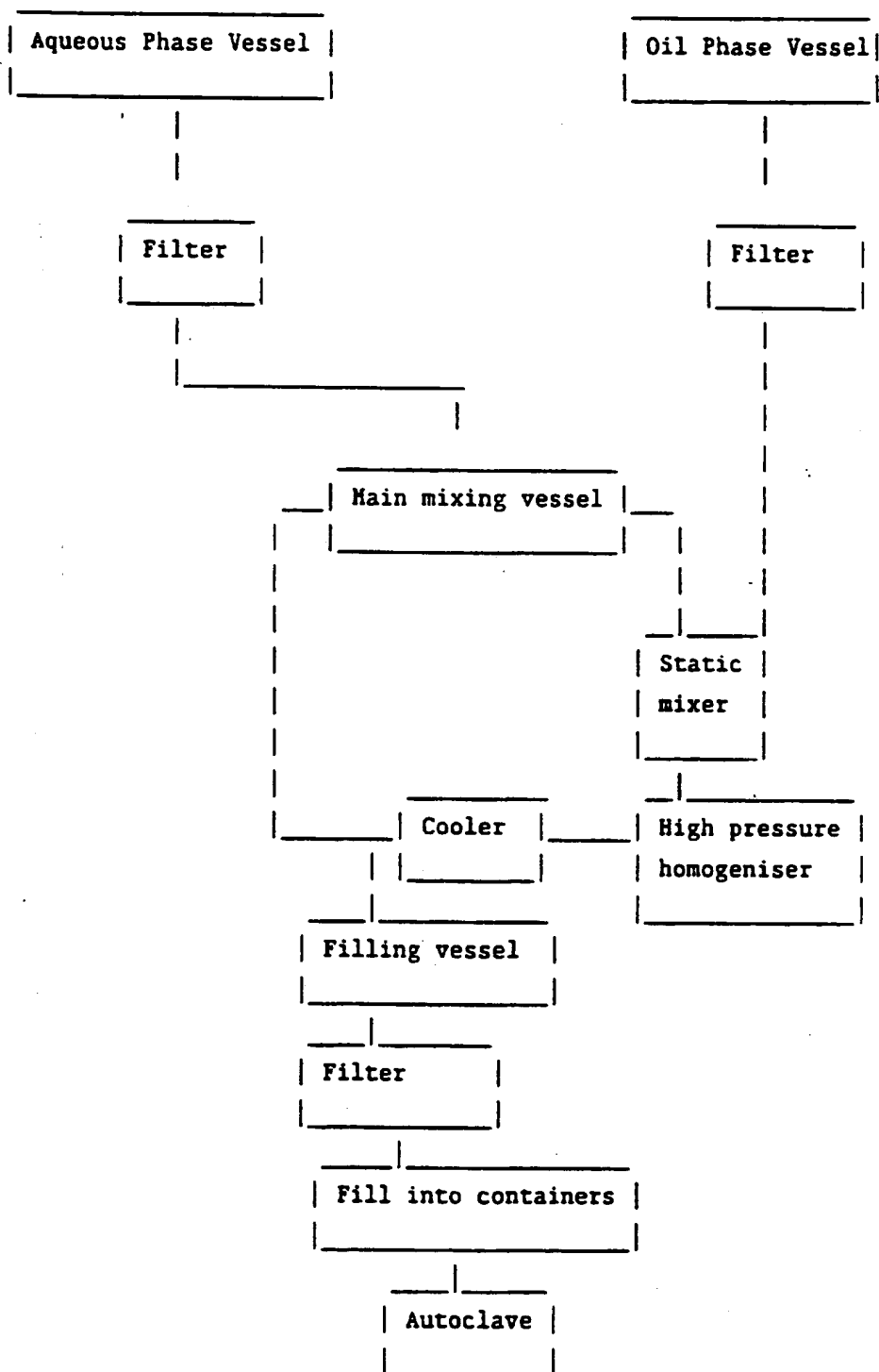
1. An aqueous phase is prepared from glycerol (2.25% by weight), disodium edetate dihydrate (0.0055% by weight), sodium hydroxide (typically  $60\text{mgL}^{-1}$ ) and Water for Injections. This mixture is stirred and taken to a temperature of approximately  $65^{\circ}\text{C}$ .
2. The aqueous phase is passed through a filter to remove particulate matter and transferred to a mixing vessel.

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3. In parallel to the above, an oil phase is prepared from soy bean oil (10.0% by weight), propofol (1.0% by weight) and egg phosphatide (1.2% by weight) in a vessel. The mixture is stirred at a temperature of approximately 75°C until all ingredients are dissolved.
4. The mixture is passed through a filter to remove particulate matter and added to the aqueous phase via a static mixer.
5. The contents of the mixing vessel are stirred and maintained at a temperature of approximately 65°C. This mixture is then circulated through a high pressure homogeniser and cooler (heat exchange system) until the required globule size [mean globule size of approximately 250 nanometres] is achieved.
6. The resultant oil-in-water emulsion is cooled and transferred into a filling vessel.
7. The emulsion is then filtered and filled into containers under nitrogen and autoclaved.

The final filtered emulsion may be filled into containers of various volumes for example ampoules (20ml), vials (50ml and 100ml) and pre-filled syringes.

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Diagram:

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An oil-in-water emulsion containing 2% (by weight) of propofol may be prepared in a similar manner using the following quantities of ingredients:

Quantities:

|                            | <u>% (weight)</u> |
|----------------------------|-------------------|
| propofol                   | 2.0               |
| soy bean oil               | 10.0              |
| egg phosphatide            | 1.2               |
| glycerol                   | 2.25              |
| disodium edetate dihydrate | 0.0055            |
| sodium hydroxide           | q.s.              |
| Water for Injections       | to 100            |



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Further oil-in-water emulsions containing 1% (by weight) of propofol may be prepared in a similar manner using the following quantities of ingredients:

Quantities:

|   | <u>% (weight)</u> | <u>% (weight)</u> |
|---|-------------------|-------------------|
| propofol                                | 1.0               | 1.0               |
| soy bean oil                            | 5.0               | -                 |
| fractionated coconut oil (Miglyol 812N) | 5.0               | 10.0              |
| egg phosphatide                         | 1.2               | 1.2               |
| glycerol                                | 2.25              | 2.25              |
| disodium edetate dihydrate              | 0.0055            | 0.0055            |
| sodium hydroxide                        | q.s.              | q.s.              |
| Water for Injections                    | to 100            | to 100            |

\* Miglyol is a trade mark

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**BIOLOGICAL ACTIVITY**

The formulations are administered parenterally to groups of 10 male mice (18-22 g) at a dose of 5-40 mg/kg. Sedation and anaesthesia are observed dependent on dose.

**MICROBIOLOGICAL ACTIVITY (COMPARATIVE)**

Formulations containing various additives were prepared by adding a concentrated aqueous solution of the additive to the commercially available oil-in-water formulation of propofol (1%) (Diprivan : Trade Mark of Zeneca Ltd). The pH of these formulations was approximately 7.5.

Broth cultures of four standard USP (United States Pharmacopeia) preservative efficacy test organisms were added to these test formulations at approximately 200 colony forming units per ml. The test formulations were incubated at 30°C and tested for viable counts after 24 and 48 hours.

**RESULTS****Formulation with sodium metabisulphite (0.1%)**

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.4                                      | 4.1             | 4.7             |
| E. coli              | 2.2                                      | 8.9             | 8.7             |
| C. albicans          | 2.8                                      | 4.4             | 7.9             |
| Ps. aeruginosa       | 2.8                                      | 4.8             | 8.9             |

Discolouration of the formulation occurred showing chemical instability.

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Formulation with sodium sulphite (0.1%)

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.8                                      | 5.7             | 6.2             |
| E. coli              | 1.6                                      | 7.8             | 8.9             |
| C. albicans          | 2.9                                      | 4.1             | 5.8             |
| Ps. aeruginosa       | 2.2                                      | 6.7             | 6.9             |

Formulation with hydroxybenzoates (0.2% methyl/0.02% propyl)

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.9                                      | 6.6             | 6.7             |
| E. coli              | 1.9                                      | 4.7             | 7.4             |
| C. albicans          | 2.8                                      | 3.0             | 3.2             |
| Ps. aeruginosa       | 2.4                                      | 2.2             | 5.8             |

Formulation with sodium calcium edetate (0.1%)

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.2                                      | 3.3             | 6.9             |
| E. coli              | 2.6                                      | <1.3            | <1.3            |
| C. albicans          | 2.9                                      | 3.1             | 3.8             |
| Ps. aeruginosa       | 2.8                                      | 6.8             | 8.2             |

Formulation with disodium edetate dihydrate (0.1%)

[pH approximately 5.5]

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 0.7                                      | 0.3             | <1.0            |
| E. coli              | 1.2                                      | 0.3             | <1.0            |
| C. albicans          | 1.0                                      | 0.8             | <1.0            |
| Ps. aeruginosa       | 1.3                                      | <1.0            | <1.0            |

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MICROBIOLOGICAL ACTIVITY (FURTHER COMPARATIVE RESULTS)

Washed suspensions of four standard USP (United States Pharmacopeia) preservative efficacy test organisms were added to these test formulations at approximately 100 colony forming units per ml. The test formulations were incubated at 25°C and tested for viable counts after 24 and 48 hours in duplicate; both results are reported.

'Diprivan' (1% propofol)

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.0                                      | 4.3             | 5.7             |
|                      | 2.0                                      | 4.6             | 5.7             |
| E. coli              | 1.7                                      | 8.1             | 7.9             |
|                      | 1.6                                      | 7.8             | 8.1             |
| C. albicans          | 1.5                                      | 2.8             | 2.6             |
|                      | 1.5                                      | 2.8             | 3.6             |
| Ps. aeruginosa       | 1.5                                      | 4.9             | 8.4             |
|                      | 1.5                                      | 3.9             | 8.0             |

Formulation with disodium edetate dihydrate (0.0055%)

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.0                                      | 1.3             | 0.5             |
|                      | 2.0                                      | 1.1             | 1.0             |
| E. coli              | 1.6                                      | 1.1             | ND              |
|                      | 1.4                                      | 1.1             | ND              |
| C. albicans          | 1.6                                      | 1.6             | 2.0             |
|                      | 1.5                                      | 1.3             | 2.1             |
| Ps. aeruginosa       | 1.6                                      | 1.0             | 0.8             |
|                      | 1.5                                      | ND              | 0.7             |

The above formulation has been further assessed against other relevant organisms.

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In a similar manner, microbiological data have been obtained for the corresponding formulation containing 2% propofol.

Intravenous fat emulsion

[comprising soy bean oil (10%), egg phosphatide (1.2%), glycerol (2.25%), sodium hydroxide (qs) and Water for Injections]

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.0                                      | 6.5             | 6.6             |
|                      | 2.0                                      | 6.6             | 6.7             |
| E. coli              | 1.5                                      | 8.0             | 8.3             |
|                      | 1.6                                      | 7.9             | 8.1             |
| C. albicans          | 1.5                                      | 1.2             | 6.0             |
|                      | 1.4                                      | 3.5             | 5.6             |
| Ps. aeruginosa       | 1.3                                      | 6.6             | 8.1             |
|                      | 1.5                                      | 6.9             | 8.1             |

Intravenous fat emulsion (as above) with disodium edetate dihydrate (0.0055%)

| <u>Test Organism</u> | <u>LOG<sub>10</sub> SURVIVORS PER ML</u> |                 |                 |
|----------------------|--|-----------------|-----------------|
|                      | <u>Zero</u>                              | <u>24 hours</u> | <u>48 hours</u> |
| S. aureus            | 2.0                                      | 1.4             | ND              |
|                      | 2.0                                      | 1.4             | ND              |
| E. coli              | 1.6                                      | ND              | ND              |
|                      | 1.5                                      | ND              | ND              |
| C. albicans          | 1.5                                      | 1.8             | 2.4             |
|                      | 1.5                                      | 2.1             | 2.2             |
| Ps. aeruginosa       | 1.6                                      | ND              | ND              |
|                      | 1.5                                      | ND              | ND              |

ND : No organisms detected on the 1ml pour plates

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The above formulation has been further assessed against other relevant organisms.

The test organisms identified above are *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231.

In a preferred embodiment the present invention provides a sterile pharmaceutical composition which comprises an oil-in-water emulsion in which propofol, dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of edetate sufficient to prevent a no more than 10-fold increase in growth of each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 for at least 24 hours as measured by a test wherein a washed suspension of each said organism is added to a separate aliquot of said composition at approximately 50 colony forming units per ml, at a temperature in the range 20-25°C, said aliquots are incubated at 20-25°C and are tested for viable counts after 24 hours.

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CLAIMS

1. A sterile pharmaceutical composition for parenteral administration which comprises an oil-in-water emulsion in which propofol, dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours after adventitious, extrinsic contamination.

2. A sterile pharmaceutical composition according to claim 1 wherein the amount of edetate is sufficient to prevent a no more than 10-fold increase in growth of clinically relevant microorganisms for at least 24 hours after contamination by up to  $10^3$  colony forming units (at a temperature in the range 20-25 C).

3. A sterile pharmaceutical composition according to claim 2 wherein the clinically relevant microorganisms are selected from strains of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Pseudomonas aeruginosa*.

4. A sterile pharmaceutical composition according to claim 1 which comprises an oil-in-water emulsion in which propofol, dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of edetate sufficient to prevent a no more than 10-fold increase in growth of each of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Candida albicans* ATCC 10231 for at least 24 hours as measured by a test wherein a washed suspensions of each said organism is added to a separate aliquot of said composition at approximately 50 colony forming units per ml, at a temperature in the range 20-25°C, said aliquots are incubated at 20-25°C and are tested for viable counts after 24 hours.

5. A sterile pharmaceutical composition according to any one of claims 1 to 4 wherein the edetate is disodium edetate.

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6. A sterile pharmaceutical composition according to any one of claims 1 to 5 which comprises up to 30% by weight of water-immiscible solvent.
7. A sterile pharmaceutical composition according to claim 6 which comprises from 10-20% by weight of water-immiscible solvent.
8. A sterile pharmaceutical composition according to any one of claims 1 to 7 wherein the water-immiscible solvent is a vegetable oil or ester of a fatty acid.
9. A sterile pharmaceutical composition according to claim 8 wherein the vegetable oil is soy bean oil.
10. A sterile pharmaceutical composition according to any one of claims 1 to 9 wherein the surfactant is a naturally occurring phosphatide.
11. A sterile pharmaceutical composition according to claim 10 wherein the phosphatide is egg phosphatide or soya phosphatide.
12. A sterile pharmaceutical composition according to any one of claims 1 to 11 wherein the pH is between 6.0 and 8.5.
13. A sterile pharmaceutical composition according to claim 12 wherein sodium hydroxide is present.
14. A sterile pharmaceutical composition according to any one of claims 1 to 13 which is isotonic with blood.
15. A sterile pharmaceutical composition according to claim 14 which is made isotonic with blood by incorporation of glycerol.
16. A sterile pharmaceutical composition according to any one of claims 1-15 which comprises from 1%-2% by weight of propofol.



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17. A sterile pharmaceutical composition according to claim 16 which contains about 1% by weight of propofol.

18. A sterile pharmaceutical composition according to claim 16 which contains about 2% by weight of propofol.

19. A sterile pharmaceutical composition for parenteral administration which comprises an oil-in-water emulsion in which propofol dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of edetate wherein the amount of edetate is a molar concentration in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ .

20. A sterile pharmaceutical composition according to claim 19 wherein the amount of edetate is a molar concentration in the range  $3 \times 10^{-5}$  to  $7.5 \times 10^{-4}$ .

21. A sterile pharmaceutical composition according to claim 20 wherein the amount of edetate is a molar concentration in the range  $1.5 \times 10^{-4}$  to  $3.0 \times 10^{-4}$ .

22. A sterile pharmaceutical composition according to claim 21 wherein the amount of edetate is a molar concentration of about  $1.5 \times 10^{-4}$ .

23. A sterile pharmaceutical composition according to any one of claims 19 to 22 wherein the source of edetate is disodium edetate.

24. A sterile pharmaceutical composition according to any one of claims 19 to 23 which comprises up to 30% by weight of water-immiscible solvent.

25. A sterile pharmaceutical composition according to claim 24 which comprises from 10-20% by weight of water-immiscible solvent.

26. A sterile pharmaceutical composition according to any one of claims 19 to 25 wherein the water-immiscible solvent is a vegetable

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oil or ester of a fatty acid.

27. A sterile pharmaceutical composition according to claim 26 wherein the vegetable oil is soy bean oil.

28. A sterile pharmaceutical composition according to any one of claims 19 to 27 wherein the surfactant is a naturally occurring phosphatide.

29. A sterile pharmaceutical composition according to claim 28 wherein the phosphatide is egg phosphatide or soya phosphatide.

30. A sterile pharmaceutical composition according to any one of claims 19 to 29 wherein the pH is between 6.0 and 8.5.

31. A sterile pharmaceutical composition according to claim 30 wherein sodium hydroxide is present.

32. A sterile pharmaceutical composition according to any one of claims 19 to 31 which is isotonic with blood.

33. A sterile pharmaceutical composition according to claim 32 which is made isotonic with blood by incorporation of glycerol.

34. A sterile pharmaceutical composition according to any one of claims 19 to 33 which comprises from 1%-2% by weight of propofol.

35. A sterile pharmaceutical composition according to claim 34 which contains about 1% by weight of propofol.

36. A sterile pharmaceutical composition according to claim 34 which contains about 2% by weight of propofol.

37. A sterile pharmaceutical composition for parenteral administration in the form of an oil-in-water emulsion which comprises:

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- a) 1% by weight of propofol,
- b) 10% by weight of soy bean oil,
- c) 1.2% by weight of egg phosphatide,
- d) 2.25% by weight of glycerol,
- e) 0.005% by weight of disodium edetate,
- f) sodium hydroxide
- g) water.

38. A sterile pharmaceutical composition for parenteral administration in the form of an oil-in-water emulsion which comprises:

- a) 2% by weight of propofol,
- b) 10% by weight of soy bean oil,
- c) 1.2% by weight of egg phosphatide,
- d) 2.25% by weight of glycerol,
- e) 0.005% by weight of disodium edetate,
- f) sodium hydroxide
- g) water.

39. A method for limiting the potential for microbial growth in a sterile pharmaceutical composition for parenteral administration which comprises the use of edetate in an oil-in-water emulsion in which propofol dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant, wherein the amount of edetate is sufficient to prevent significant growth of microorganisms for at least 24 hours after adventitious, extrinsic contamination.

40. A method for limiting the potential for microbial growth in a sterile pharmaceutical composition for parenteral administration which comprises the use of edetate in an oil-in-water emulsion in which propofol dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant, wherein the amount of edetate is a molar concentration in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ .

41. A method of producing anaesthesia in a warm-blooded animal which

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comprises administering an effective amount of a sterile pharmaceutical composition according to any one of claims 1 to 18.

42. A method of producing anaesthesia in a warm-blooded animal which comprises administering an effective amount of a sterile pharmaceutical composition according to any one of claims 19 to 36.

43. A method of producing anaesthesia in a warm-blooded animal which comprises administering an effective amount of a sterile pharmaceutical composition according to any one of claims 37 or 38.

44. A sterile pharmaceutical composition for parenteral administration which comprises an oil-in-water emulsion in which propofol is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours after adventitious, extrinsic contamination.

45. A sterile pharmaceutical composition for parenteral administration which comprises an oil-in-water emulsion in which propofol is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of edetate wherein the amount of edetate is a molar concentration in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ .

46. A method for limiting the potential for microbial growth in a sterile pharmaceutical composition for parenteral administration which comprises the use of edetate in an oil-in-water emulsion in which propofol is emulsified with water and stabilised by means of a surfactant, wherein the amount of edetate is sufficient to prevent significant growth of microorganisms for at least 24 hours after adventitious, extrinsic contamination.

47. A method for limiting the potential for microbial growth in a sterile pharmaceutical composition for parenteral administration which comprises the use of edetate in an oil-in-water emulsion in which propofol is emulsified with water and stabilised by means of a

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surfactant, wherein the amount of edetate is a molar concentration in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ .

48. A method of improving the time for administration, and/or the time between the changes of giving sets, for an oil-in-water emulsion of propofol by including in said emulsion an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours after adventitious, extrinsic contamination.

49. A method of improving the time for administration, and/or the time between the changes of giving sets, for an oil-in-water emulsion of propofol by including in said emulsion edetate in a molar concentration in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ .

50. A sterile, aqueous composition for parenteral administration which comprises an oil-in-water emulsion which is emulsified with water and stabilised by means of a surfactant and which further comprises an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours after adventitious, extrinsic contamination.

51. A sterile, aqueous composition for parenteral administration which comprises an oil-in-water emulsion which is emulsified with water and stabilised by means of a surfactant, and which further comprises an amount of edetate wherein the amount of edetate is a molar concentration in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ .

52. A sterile pharmaceutical composition which comprises an oil-in-water emulsion containing a therapeutic or pharmaceutical agent, in which the agent, either alone or dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant and which further comprises an amount of edetate sufficient to prevent significant growth of microorganisms for at least 24 hours after adventitious, extrinsic contamination.

53. A sterile pharmaceutical composition which comprises an

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oil-in-water emulsion containing a therapeutic or pharmaceutical agent, in which the agent, either alone or dissolved in a water-immiscible solvent, is emulsified with water and stabilised by means of a surfactant and which further comprises an amount of edetate wherein the amount of edetate is a molar concentration in the range  $3 \times 10^{-5}$  to  $9 \times 10^{-4}$ .

54. A composition according to claim 52 comprising an antifungal agent, anaesthetic, antibacterial agent, anti-cancer agent, anti-emetic, agent acting on the central nervous system, steroid, barbiturate or a vitamin preparation.

55. A composition according to claim 53 comprising an antifungal agent, anaesthetic, antibacterial agent, anti-cancer agent, anti-emetic, agent acting on the central nervous system, steroid, barbiturate or a vitamin preparation.

# INTERNATIONAL SEARCH REPORT

Internat. J. Application No  
PCT/GB 95/00579

|   |  |  |
|---|--|--|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC 6    A61K31/05    A61K9/107    A61K47/18  |  |  |
| According to International Patent Classification (IPC) or to both national classification and IPC   |  |  |
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC 6    A61K   |  |  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched   |  |  |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)  |  |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>   |  |  |
| Category *  | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.  |
| X   | FR,A,2 265 357 (I.C.I. LTD) 24 October 1975<br>cited in the application<br>see page 2, line 5 - line 10<br>see page 3, line 3 - line 9<br>see page 4, line 14<br>see page 5, line 4 - line 5<br>see page 6 - page 7; examples 2,3<br>---           | 1-6,<br>12-14,<br>16-18  |
| Y   | BR. J. ANAESTH.,<br>vol. 68, no. 2, February 1992 LONDON,<br>pages 193-197,<br>NAKAMURA K. ET AL 'Direct vasoconstrictor<br>and vasodilator effects of propofol in<br>isolated dog arteries'<br>see page 194, column 2, paragraph 2<br>---<br>-/-- | 1-47,<br>50-55   |
| <div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.</span> <span><input checked="" type="checkbox"/> Patent family members are listed in annex.</span> </div>   |  |  |
| <div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div> |  |  |
| Date of the actual completion of the international search<br><br><div style="text-align: center; font-weight: bold;">2 August 1995</div>  |  | Date of mailing of the international search report<br><br><div style="text-align: center; font-weight: bold;">22. 08. 95</div> |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+31-70) 340-3016   |  | Authorized officer<br><br><div style="text-align: center; font-weight: bold;">Boulois, D</div>                                 |

## INTERNATIONAL SEARCH REPORT

Internat. J. Application No.

PCT/GB 95/00579

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|------------|--|-----------------------|
| Y          | INDIAN J. PHARMACY,<br>vol. 27, 1965<br>pages 147-148,<br>PATEL R.P. ET AL 'Disodium salt of EDTA<br>as an antimicrobial agent'<br>see the whole document<br>---   | 1-47,<br>50-55        |
| A          | BR. J. ANAESTH.,<br>vol. 66, no. 1, January 1991 LONDON,<br>pages 66-72,<br>PERRY S. MOUTON ET AL 'Effect of<br>anaesthesia with propofol on drug<br>distribution and metabolism in the dog'<br>see page 66, column 2, paragraph 1<br>---                                    | 1-47,<br>50-55        |
| A          | WO,A,90 06055 (UNILEVER PLC) 14 June 1990<br><br>see page 5, line 5 - line 9; claim 9<br>---   | 1-47,<br>50-55        |
| A          | CHEMICAL ABSTRACTS, vol. 113, no. 10,<br>3 September 1990<br>Columbus, Ohio, US;<br>abstract no. 84881,<br>MORITA T. ET AL 'Ophthalmic solutions<br>containing benzalkonium chloride,<br>p-hydroxybenzoate esters and chelating<br>agents'<br>see abstract<br>& DATABASE WPI | 1                     |
| A          | Section Ch, Week 9020<br>Derwent Publications Ltd., London, GB;<br>Class B05, AN 90-151812<br>& JP,A,02 096 515 (SANTEN PHARM KK) , 9<br>April 1990<br>see abstract  | 1                     |
| A          | & PATENT ABSTRACTS OF JAPAN<br>vol. 14 no. 294 (C-732) ,26 June 1990<br>& JP,A,02 096515 (SANTEN PHARM CO LTD) 9<br>April 1990,<br>see abstract<br>-----   | 1                     |



# INTERNATIONAL SEARCH REPORT

I tational application No.

PCT/GB95/00579

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 48, 49  
because they relate to subject matter not required to be searched by this Authority, namely:  
Method of treatment of the human body by therapy (see rule 39(1v)PCT).
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/GB 95/00579

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| FR-A-2265357                              | 24-10-75            | GB-A- 1472793              | 04-05-77            |
|   |                     | AU-B- 502164               | 12-07-79            |
|   |                     | AU-A- 7953675              | 30-09-76            |
|   |                     | BE-A- 827290               | 29-09-75            |
|   |                     | CA-A- 1038764              | 19-09-78            |
|   |                     | DE-A- 2513797              | 09-10-75            |
|   |                     | JP-A- 50154410             | 12-12-75            |
|   |                     | NL-A- 7503696              | 30-09-75            |
|   |                     | SE-A- 7503542              | 29-09-75            |
|   |                     | US-A- 4798846              | 17-01-89            |
|   |                     | US-A- 4056635              | 01-11-77            |
|   |                     | US-A- 4452817              | 05-06-84            |
| WO-A-9006055                              | 14-06-90            | AU-B- 636591               | 06-05-93            |
|   |                     | AU-A- 4659989              | 26-06-90            |
|   |                     | JP-T- 3503171              | 18-07-91            |